

FORM PTO-1590 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER: BO 42517 JGD 097957349 <small>CLASS. BY 42517 JGD/1.5</small>
INTERNATIONAL APPLICATION NO.: PCT/NL00/00200	INTERNATIONAL FILING DATE: 24 MARCH 2000 (24.03.00)	PRIORITY DATE CLAIMED: 24 MARCH 1999
TITLE OF INVENTION: METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES		
APPLICANT(S) FOR DO/EO/US: Edwin Johannus Gerardus DE WINTER, Hendrikus Cornelis MASTWIJK and Paul Vincent BARTELS		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1.	<input checked="" type="checkbox"/>	This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2.	<input type="checkbox"/>	This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3.	<input checked="" type="checkbox"/>	This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4.	<input checked="" type="checkbox"/>	A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5.	<input checked="" type="checkbox"/>	A copy of the International Application as filed (35 U.S.C. 371(c)(2))
	<input checked="" type="checkbox"/>	a. is transmitted herewith (required only if not transmitted by the International Bureau).
	<input type="checkbox"/>	b. has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
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6.	<input type="checkbox"/>	A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7.	<input checked="" type="checkbox"/>	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
	<input checked="" type="checkbox"/>	a. are transmitted herewith (required only if not transmitted by the International Bureau).
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8.	<input type="checkbox"/>	A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9.	<input checked="" type="checkbox"/>	An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10.	<input type="checkbox"/>	A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
Item 11. to 16. below concern document(s) or information included:		
11.	<input checked="" type="checkbox"/>	An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12.	<input checked="" type="checkbox"/>	An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13.	<input checked="" type="checkbox"/>	A FIRST preliminary amendment.
	<input type="checkbox"/>	A SECOND or SUBSEQUENT preliminary amendment.
14.	<input type="checkbox"/>	A substitute specification.
15.	<input type="checkbox"/>	A change of power of attorney and/or address letter.
16.	<input checked="" type="checkbox"/>	Other items or information: INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT/PEPA/409), INTERNATIONAL SEARCH REPORT (PCT/ISA/210), APPLICATION DATA SHEET, ABSTRACT

U.S. APPLICATION NO.

09/937349

INTERNATIONAL APPLICATION NO.
PCT/NL00/00200ATTORNEY'S DOCKET NO.
BO 42517 JGD17. ☒ The following fees are submitted:**BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$ 1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$ 690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$ 100.00

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\$ 860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$
Total claims	8 - 20 =	0	X \$18.00	\$
Independent claims	1 - 3 =	0	X \$80.00	\$
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ \$270.00	\$

TOTAL OF ABOVE CALCULATIONS =

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Reduction of 1/2 for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +

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TOTAL NATIONAL FEE =

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September 24, 2001

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09/937349

JCOB Rec'd PCT/PTO 24 SEP 2001

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Edwin DE WINTER et al.

Serial No. (unknown)

Filed herewith

METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to the first Official Action and calculation of the filing fee, please substitute Claims 1-10 as originally filed, which appear on pages 14-15, with Claims 1-11 as filed in the Article 19 amendment of September 18, 2000. Thereafter, please replace claims 1-11 with claims 1-7 as in the Article 34 Amendment. The pages containing Claims 1-7 are marked "AMENDED CLAIMS" and are attached hereto. Following the insertion of Claims 1-7, please amend these claims as follows:

IN THE CLAIMS:

Please amend claims 4-7 as follows:

--4. (Amended) Method for treating products according to claim 1, characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.

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5. (Amended) Method according to claim 1, characterised in that dependent on the type of product and target organisms contained in the product, the maximum field strength during a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target micro-organisms and or spores are functionally affected or inactivated leading to into a shelf life stable, microbiologically safe product.

6. (Amended) Method according to claim 1, characterised in that dependent on the type of product and the types of cellular structures contained in the product, the maximum field strength reached in a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds with the bulk product.

7. (Amended) Method according to claim 1, characterised in that the maximum field strength in the product during each pulse, the repetition frequency, the number of cycles and the total residence time in the process are selected such that the temperature of the product does not exceed a predetermined value during treatment.--

Edwin DE WINTER et al.

R E M A R K S

The above changes in the claims merely place this national phase application in the same condition as it was during Chapter II of the international phase, with the multiple dependencies being removed from claims 4-7. Following entry of this amendment by substitution of the pages, only claims 1-7 remain pending in this application. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE".

Respectfully submitted,

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September 24, 2001

AMENDED CLAIMS

[received by the International Bureau on 18 September 2000 (18.09.00);
original claims 1-10 replaced by amended claims 1-11 (2 pages)]

1. Method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in
5 a treatment device comprising two electrodes connected to an electronic circuit such that in said device and in said product an electrical field in excess of 500 V/cm is created, provided that the rise time or the trailing edge of each imposed voltage pulse is shorter than the associated electronic relaxation time of the product defined by the electrical properties of the product.
- 10 2. Method for treating products according to claim 1, where the cellular structures present in the bulk product are pathogenic or spoilage organisms and/or their spores, where treatment is applied as a mild preservation method to prevent the outgrowth of such organisms in the product after production during distribution or storage.
- 15 3. Method for treating products according to claim 1, where the cellular structures present or the bulk product contains certain compounds which are exchanged at a higher rate through the membrane of such structures when applying the treatment.
- 20 4. Method for treating products according to claim 1, characterised in that the trailing edge of each pulse starts within a time span shorter than the relaxation time of the product.
- 25 5. Method for treating products according to claim 1 or 2, characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.
- 30 6. Method according to any of the preceding claims, characterised in that dependent on the type of product and the types of micro-organisms contained in the product, the maximum field strength during a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target micro-organisms and or spores are functionally affected or inactivated leading to a shelf life stable, microbiologically safe product.

7. Method according to any of the preceding claims, characterised in that dependent on the type of product and the types of cellular structures contained in the product, the maximum field strength reached in a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds with the bulk product.
8. Method according to one of the preceding claims, where the imposed voltage on the treatment device is produced at a single frequency or at multiple distinct frequencies using electronic circuitry at frequencies in the range of 100 Hz to 10 GHz.
9. Method according to one of the preceding claims, where the imposed voltage on the treatment device is produced at a single frequency or at multiple distinct frequencies using resonant electronic structures at frequencies in the range of 100 Hz to 10 GHz.
10. Method according to any of the preceding claims, characterised in that the maximum field strength of each pulse, the repetition frequency, the number of cycles and the total residence time during a treatment are selected such that the temperature of the product does not exceed a predetermined value during treatment.
11. Method according to any of the preceding claims, characterised in that the temperature of the product is maintained below 121 degrees centigrade.

13.03.2001

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CLAIMS

1. Method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in a treatment device comprising two electrodes connected to an electronic circuit such that in said product an electrical field is created, characterized in that the rise time or the leading edge of each imposed voltage pulse is shorter than the associated electronic relaxation time of the product under treatment which is defined by the ratio of electrical conductivity and permittivity of the product.

2. Method for treating products according to claim 1, where the cellular structures present in the bulk product are pathogenic or spoilage organisms and/or their spores, where treatment is applied as a mild preservation method to prevent the outgrowth of such organisms in the product after production during distribution or storage.

3. Method for treating products according to claim 1, where the cellular structures present or the bulk product contain target compounds as e.g. minerals, enzymes or molecular compounds which are exchanged at a higher rate through the membrane of such structures when applying the treatment.

4. Method for treating products according to the preceding claims, characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.

5. Method according to any of the preceding claims, characterised in that dependent on the type of product and target organisms contained in the product, the maximum field strength during a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target micro-organisms and or spores are functionally affected or inactivated leading into a shelf life stable, microbiologically safe product.

6. Method according to any of the preceding claims, characterised in that dependent on the type of product and the types of cellular structures contained in the product, the

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maximum field strength reached in a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds with the bulk product.

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7. Method according to any of the preceding claims, characterised in that the maximum field strength in the product during each pulse, the repetition frequency, the number of cycles and the total residence time in the process are selected such that the temperature of the product does not exceed a predetermined value during treatment.

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"VERSION WITH MARKINGS TO SHOW CHANGES MADE"

Claims 4-7 have been amended as follows:

4. ~~(Amended)~~ Method for treating products according to ~~the preceding claims, claim 1,~~ characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.

5. ~~(Amended)~~ Method according to ~~any of the preceding claims, claim 1,~~ characterised in that dependent on the type of product and target organisms contained in the product, the maximum field strength during a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target micro-organisms and or spores are functionally affected or inactivated leading to into a shelf life stable, microbiologically safe product.

6. ~~(Amended)~~ Method according to ~~any of the preceding claims, claim 1,~~ characterised in that dependent on the type of product and the types of cellular structures contained in the product, the maximum field strength reached in a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds with the bulk product.

7. ~~(Amended)~~ Method according to ~~any of the preceding claims, claim 1,~~ characterised in that the maximum field strength in the product during each pulse, the repetition

frequency, the number of cycles and the total residence time in the process are selected such that the temperature of the product does not exceed a predetermined value during treatment.

METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES

INTRODUCTION

The invention relates to a method for treating products, which may contain
5 cellular material of eukaryotic and or prokaryotic origin, in particular micro-organisms,
located in a device comprising two electrodes onto where voltage cycles are imposed
by an auxiliary electric source such that in the device and in the product electrical fields
are created for a short period of time.

A prior art method of this type is known as the Pulsed Electric Field (PEF)
10 process.

PRIOR ART

Many biological systems, such as micro-organisms, comprise a cell membrane to
regulate its energy balance. Cell membranes consist of a lipid double layer whereby the
15 lipids are made of a polar head and a fatty acid tail. Metabolic processes are regulated
by said cell membrane. Physical damage of the cell membrane may lead to inactivation
of the system or to an increase of the exchange of mass transport through the membrane
such as inter-cellular material and/or compounds present in the bulk of the product. In
the case of micro-organisms damage to the cell membrane may lead to inactivation of
20 the organism such that the cell division process will be interrupted or its functional
abilities to produce metabolic compounds is affected.

Damage to the cell membrane of micro-organisms may be caused by bringing the
micro-organisms into a high electric field. An sufficiently high externally imposed
potential difference across the micro-organism is believed to lead to damage of the cell
25 membrane as it leads to the inactivation of the micro-organisms as such. A treatment
based on PEF can be performed by using a pulsed DC voltage source. The above
mentioned PEF process relies on the use of high voltage pulses to generate a pulsating
electric field of in a product of such a short duration that the heating of the bulk product
is restricted.

A very simple system in which the PEF-method is applied is described in
30 US5393541 and US5447733. Both related publications illustrate a system comprising
a container which is filled by product to be treated and a metal electrode which is
lowered into the container. The container itself forms the other electrode and both

electrodes are connected to a power supply delivering pulses of at 2 kV or more with a duration of typically two microseconds.

Another embodiment of a chamber for treating fluid products according to the PEF-method is described in US4695472 and US4838154. In this embodiment two flat electrodes are positioned opposite each other with a flow channel in between. Both electrodes are connected to a power source which during operation generates pulses. In this configuration a pulsed electrical field is produced within the product inside the channel in agreement with the PEF-method. As described, in both patents the product is subjected to high electric field pulses each having a minimum field strength of at least 5 kV/cm and each having a duration of at least about one micro-second. Preferably a duration in the range from about 5 to about 100 micro-seconds.

A further example of a system in which a PEF-method is performed is described in US5235905, US5776529 and an article with the title "Inactivating Micro-organisms Using a Pulsed Electric Field Continuous Treatment System" by Bai-Lin Qin published in IEEE Transactions on Industrial Applications, Volume 34, nr. 1, 1 February 1998, pages 43/49. This prior art system comprises a so-called coaxial treatment chamber. During operation electrical pulses are supplied to both electrodes such that electrical field strengths in the range of 35 to 55 kV/cm are developed. Preferred pulse duration's are less than 100 milliseconds, more preferably in the range of 0.1 to 100 microseconds and even more preferably in the range of approximately 0.2 to 10 micro-seconds.

A system comprising a series of tubular treatment chambers is described in US5690978. Each chamber has electrically conducting end-sections, which act as electrodes separated by a non-conducting intermediate section. During operation a pulsed electric field is developed in the treatment chamber with a typical pulse duration time of three microseconds at an applied electric field strength of $E = 30$ kV/cm whereas the temperature reaches a maximum $T = 36^\circ$ C.

In all these prior art systems the medium to be treated has to be in physical and electrical contact with both the electrodes during the treatment.

A different mode of treatment is described in an article with the title "Inactivation of *Yersinia enterocolitica* Gram-Negative Bacteria using high voltage pulse technique" by Piotr, Lubicki et al published as record of the industry application conference (IAS, Orlando, October 9/12, 1995, Volume 2, Number 30, pages 1338-1344, Institute of

Electrical and Electronics Engineers ISBN 0-7803-3009/9, page 1339, column 1, lines 3-24). In this article a treatment device is described comprising a cylindrical electrode system including a rod shaped inner electrode inside a cylindrically shaped outer electrode. The inner electrode is connected to a source of high voltage pulses and the outer electrode is electrically grounded. The product to be treated is contained between both electrodes in a helical shaped glass tube and the remaining space within the electrode system is filled with water.

During operation a pulsed electrical field is developed between the electrodes where the rise time of each pulse is between 500 and 1300 nanoseconds and the voltage has a peak value equal to 45, 60 or even 75 kV. The article, however, does not provide any information about the electrical field strength within the product to be treated nor the processing temperatures of the product. In the article it is stressed that "in order to cause electroporation of a cell membrane, the voltage magnitude must be high enough to induce suitable value of transmembrane potential for breakdown of the membrane, and at the same time, duration of the voltage pulse must be at least higher then the relaxation time of a bacteria suspension". The product to be treated in the described model is a solution of NaCl in water for which $\epsilon = 0.7 \text{ nF/m}$ with an electrical conductivity between 0.8 and 1.2 S/m. The relaxation time is therefore between 0.6 and 0.9 nanoseconds. In other words, the above mentioned rise time of 500 to 1300 ns is indeed significantly larger than the relaxation time of the product to be treated. It is furthermore indicated that "there is no remarkable effect of increasing rise time within the range of 500 to 1300 ns".

OBJECT OF THE INVENTION

An objective of the invention is now to provide another method for treating suitable products, which may contain micro-organisms by developing pulsed electrical fields within the product by a different coupling. More specific it is an objective of the invention to provide a method for mild preservation of products where direct contact between the product and the electrodes is not required and where a different phenomenon is exploited to produce a substantial electrical field inside a product.

THE INVENTION

In agreement with these objectives, the invention now provides a method for

treating products by bringing the product into a treatment device containing two electrodes onto which a rapidly changing high voltage difference is imposed. The two electrodes are connected to an electronic circuitry such that the device and the product are subjected to a time dependant voltage. The time dependence of the imposed voltage is primarily characterised by the rise time of the voltage which is in duration shorter than the so-called relaxation time of the product. The relaxation time has to be understood as the time necessary to obtain a complete separation of charges in a product from the moment an external voltage difference is induced over a product column. The charges in a food product may be the result of a mineral salt content of e.g. NaCl or KCl. In foodstuffs of sufficiently high water content the NaCl molecules are dissolved as Na⁺ and Cl⁻ ions. The relaxation time can be expressed as $\tau = \epsilon/\sigma$ whereby σ is the electrical conductivity of the fluid and ϵ is the dielectric constant or permittivity.

Dynamical Polarisation Process

This method according to the invention, called the Dynamic Polarisation Process or DPP method, is based on the insight that foodstuffs and bio-mass in general are neither not very good conductors nor insulators. Typically, the electrical conductivity of high water content foodstuffs range from 0.1 S/m to 10 S/m and the permittivity is close to the permittivity of water i.e. 0.71 nF/m. As a result, a product column that is initially polarised by an external imposed voltage difference, will lose its polarisation after 0.07 to 7 nanoseconds. In this application this impulse response is exploited as follows: if an electrical voltage is imposed sufficiently fast by means of an external source, an electrical field will be present inside the product for a duration equal to the relaxation time. As soon as a pre-determined maximum peak amplitude is obtained, the external imposed voltage is allowed to vanish. Thus, it is only necessary to reach a maximum required voltage in order to induce a voltage gradient within a product for a certain period of time. The treatment can be applied several times by allowing more cycles as described previously. The level of the required voltage difference needed (or electrical field strength) in a particular application depends on the type of bulk medium, the micro-organisms under consideration and the number of cycles. Note, that micro-organisms that are present in the product will be affected by the voltages cycles as well. As the dynamical polarisation process is distinctly different from the PEF process, the

interaction with organisms is of a different origin. As no charge is displaced in case of DPP and the coupling to the product is capacitive the inactivation of rigid micro structures as bacterial spores may be possible as well.

It is preferred that the described DPP method is performed under circumstances whereby the trailing edge of each pulse has ended within the relaxation time of the product. Under these circumstances any electrical current due to movement of charges is prevented even if there is a physical and or electrical contact between the electrodes and the product. In general, the DPP process can be applied e.g. in continuous flow where product is pumped through a device in which treatment takes place during its residence. Product preparation, treatment and after handling of foodstuffs, pharmaceuticals etc. can be similar to systems where heat pasteurisation/sterilisation or PEF treatment are applied in continuous flow. The exception is that treatment is employed at DPP conditions.

In case of batch operation it is preferred that there is no direct contact between the electrodes and the product. In this case the electrodes in a treatment device can be the plates of a capacitor configuration and the product just has to be present between said plates. In such an embodiment of the method, the product is not confined to a specific tube, channel etc. defined by the treatment device itself but the product is present e.g. within a suitable package which is placed into the device. Examples of products that may be treated in batch are pouches, boxes, containers but also complete eggs in shell. These products can be treated in a (semi-) continuously fashion. For this an controlled automated system may be used comprising a conveyor belt and a treatment device which is connected to suitable electronic source to apply the DPP method.

FIGURES

In the following part of this specification the invention will be described in more detail with reference to the attached drawings.

Figure 1a illustrates a first principle embodiment of a semi-continuous or batch operated device for performing the method according to the invention.

Figure 1b illustrates a second principle embodiment of a device for performing the method according to the invention in continuous flow.

Figure 2a illustrates schematically a pulse series applied in a typical PEF process.

Figure 2b illustrates schematically a pulse series applied in a process according to the invention.

DETAIL DESCRIPTION

The apparatus in figure 1a comprises two electrically conducting plates, 10 and 12, which together form a capacitor configuration. Both plates are connected through the respective wires 18 and 20 to a electrical power source 22. Between the plates a packed product 14 is positioned. Typically, the packaging material is made out of electrical insulating material e.g. plastic, glass or carton which contains the product to be treated.

The apparatus in figure 1b comprises two electrically conducting plates, 30 and 32, which configure a capacitor configuration. Both plates are connected through the respective wires 38 and 40 to a electrical power source 42. Between the plates a conduit 34 is installed through which the product is treated in continuous flow. Typically, the conduit is made out of electrical insulating material. This conduit is part of the treatment device.

In figure 1a a product is positioned between the plates 10, 12. The product may for instance be transported and loaded in the device by a conveyer belt 17. In figure 1b there is contact between the plates 30, 32 and the conduit 34. The conduit is part of the device. It is supposed that this conduit is made of electrically insulating material. For treating the product 16 respectively 36 the source 22 respectively 42 provides high voltage pulses with a properly defined rise time and peak voltage. An example of a suitable pulse cycle is illustrated in figure 2a. The illustrated pulses are characterised by a rising edge 52, a short section 54 at maximum voltage level and a trailing edge 56.

If a pulse with a proper rise time is supplied to a capacitor configuration as illustrated by figure 1a or 1b an electric field is generated instantaneously within the product by means of molecular polarisation. Momentarily an electrical field is present within the product. This will induce an ion migration process within the product to be treated. Therefore, an electrical field due to DPP can exist only temporarily. After a time period τ this field will be eliminated by the ion displacement. At this point the electrical field within the product is cancelled. This short period of time, also known as the relaxation time, is dependent on the electrical conductivity σ of the product and the dielectric constant ϵ according to: $\tau = \epsilon/\sigma$.

In case the externally imposed voltage pulse has a rise time which is shorter then the above-mentioned relaxation period the molecular polarisation will temporarily cause an electric field across the product and therewith across the biological cells within the product. If the strength of this induced field is sufficient, this probably leads to damaging of membrane structures present. Due to the non-stationary nature of this mechanism the time dependence has to be considered. In this situation no stationary electric current is exploited as in the case of the PEF process.

Heat dissipation

An amount of heat will be developed as result of dielectric losses caused by molecular rotation and ionic losses. As pointed out previously, the rise time τ_r of the imposed pulse illustrated in figure 2a has to fulfil the requirement $\tau_r < \tau$. The top section of the pulse is preferably selected very small to avoid any further migration process as soon as the maximum voltage level is reached. That implies that preferably the sum of the periods τ_r and τ_p has to be smaller than τ .

Apart from the rise time, the maximum voltage level V_m obtained at the end of the rise time has to be selected as well. This level is dependent on the type of product, kind and thickness of packaging materials and the configuration of the treatment device. In general, the peak voltage should be such that a sufficiently high electrical field strength is reached inside the product under consideration. Typically field strengths in excess of 1 kV/cm should be employed. The number of polarisation cycles needed and the time lag between them depends on what energy input is required and what the maximum temperature is that during the process can be allowed. In practice not one but a larger number of pulses will be needed to obtain a more intense treatment.

To clearly indicate the difference in the pulse characteristics used in the PEF process on the one hand, and the process according to underlying application on the other hand figure 2b is added. On the same time axis a typical PEF pulse and a typical DPP pulse are illustrated for the treatment of an assumed high water content product with a electrical conductivity of 1 S/m. The following values are typically required:

	PEF	DDP
rising edge	100 nsec	0.7 nsec
steady state	2000 nsec	<0.7 nsec
trailing edge	100 nsec	<0.7 nsec.
5 maximum imposed electrical field strenght (inside product)	30 kV	30 KV

DPP in practice

In a practical test the method discussed in this application was exercised by

10 stopping the lactic acid production by Lacto bacilli strains. In foodstuffs this kind of organisms are known to cause spoil food during storage by acidification, thereby limiting the shelf life during storage. A small amount of fresh yoghurt, dissolved in demineralised water, was used as a model product. The inoculation of the samples was approximately 10^5 organisms per millilitre at a pH=7.0. The conductivity of the

15 inoculated buffer was such that a relaxation time in excess of 100 ns was obtained for one part of the batch and a relaxation time of much less than 100 ns for a second part of the batch. The two different stocks of model product were distributed over 64 bags, made out of plastic film bags (Stomach). The tickness of the bags was 100 micrometer and the permittivity of the material is 270 nF/m. The bags were hermetically sealed

20 allowing a minimal head space of air as a result of the sealing procedure. Part of this batch was non-treated as a reference, a second part was treated by conventional heat (pasteurised) at 80 degrees centigrade for 10 minutes. A third part of the batch was treated at different DPP conditions and at intensity levels. This included the cases where either the conditions where $\epsilon/\sigma > \text{pulse rise time}$ and $\epsilon/\sigma < \text{pulse rise time}$. In

25 other words, several different control experiments have been taken into account to evaluate the validity of the claims in this patent application.

A custom-made high voltage power supply was used to produce voltages cycles over more than 15 kV within 100 ns. In the demonstration set-up the film bags where provided with adhesive aluminium foil at the outer side. The film bags loaded into a

30 device similar as described in figure 1a. Note that as the bags are made out of plastic, which is an good electric insulator. no charge can flow through the product.

After treatment the samples were added to a sterile sample of milk at pH 7.0 which were incubated at 40 degrees centigrade for 12 hours hereafter. After this time,

the pH of the samples was measured to check whether acidification had occurred or not. A reading of $\text{pH} < 5.0$ was considered as acidified or spoiled whereas levels of $\text{pH} > 6.8$ were considered as non-spoiled. The result of these treatments can be found in table 2. In case of the non-treated samples all of the 17 samples demonstrated spoilage.

For the chosen level of pasteurisation 16 bags out of 23 were successfully heat pasteurised. For 7 bags spoilage could be demonstrated indicating that some *Lacto Bacilli* survived pasteurisation at this temperature-time combination. For the DPP processed bags the electric field strength of the treatment have to be sufficient intense as well as the total treatment time. The total treatment time is defined by the relaxation time multiplied by the number of voltage cycles employed. Furthermore, the necessity of the required condition that $\epsilon/\sigma > \text{pulse rise time}$ is demonstrated. In treatment G in table 2, the proper conditions were found to completely stop the acidification. In this case 8 out of 8 treatments has led to a full suppression of the spoilage. This was achieved at a maximum product temperature of 40 degrees centigrade. In addition, the total processing time needed is much less than the required time for heat pasteurisation at 80 degrees centigrade.

As show in table 1, in all cases the temperature increase was restricted to small values which in general are not obtainable with prior art methods.

Table 1 Measured temperature increments for various treatments. The ambient temperature was 20 degrees centigrade.

	Treatment	Treatment Period	Field strength	Temperature
		(ms)	(kV/cm)*	Increase (°C)
25	A	2	20	0.5
	B	20	20	1.0
	C	200	20	8.5
	D	200	25	18.0

(*) Within the product. This value is due to the instantaneous polarisation (DPP process) and is determined by finite element analysis modelling of the total system. The field strength is evaluated at the maximum applied voltage.

In table 2 the inactivation of Lacto Bacilli in sealed bags and treated with the DPP process is compared with a conventional heat treatment:

5 Table 2.

	Treatment (time)	relaxation time of product	E (kV/cm)*	number of bags	%
	None	< > 100 ns	-	17	100%
	heat pasteurisation	< > 100 ns	-	23	30%
10	E (2 ms)	< > 100 ns	20	4	100%
	F (200 ms)	< > 100 ns	13	12	100%
	G (200 ms)	> 100 ns	20	8	0%

(*) Within the product. This value is due to the instantaneous polarisation (DPP

15 process) and is determined by finite element analysis modelling of the total system. The field strength is evaluated at the maximum applied voltage.

Characteristics

20 In the following the typical characteristics of both PEF and DPP are shortly resumed:

In a typical PEF process pulses of the type illustrated in figure 2b are applied. During a PEF-treatment the product to be treated is in contact with two conducting electrodes which are connected to a pulsed power source. By means of said power source an stationary electric field is imposed according to $j = \sigma E$. Typically the pulses, 25 applied to said electrodes are maintained for some microseconds. Under these conditions a stationary situation is obtained in terms of the electric parameters current and voltage. That is an electric current density by movement of charge is required to sustain a net electric field. This is by transport of e.g. ions dissolved in the product which are dragged to the product during the actual treatment.

30 One or more of the beneath indicated characteristics are typical for PEF-treatment:

(1) there is a continuous supply of electric current during the pulse. This current is delivered by a capacitor in an electronic pulse circuit whereby a so-called pulse-

forming network (PFN) may be used to maintain the voltage across the treatment device constant while the discharging the capacitor. If no PFN is applied the voltage will decay exponentially in time. The time constant RC of this decay is related to the capacity (C) of the charged capacitor and to the ohmic resistance R of the electronic circuit;

(2) the electronic circuit supplies a peak power which can be related to $P = \sigma E^2 V$ whereby E is the spatial average of the field strength applied, σ the electric conductivity of the product and V is the total volume of the product being treated;

(3) the pulse is preferably of rectangular shape, in other words: the voltage is kept to a constant level during the duration of the pulse;

(4) a typical pulse duration of some microseconds is applied;

(5) the electrodes are in physical contact with the medium to be treated;

(6) the average dissipated power $\langle P \rangle$ is given by $\langle P \rangle = \sigma E^2 t j$ whereby t is the total treatment time and j is the product throughput.

(7) the heat development in the PEF process is determined by Ohmic heating and is given by $\langle P \rangle = \langle U \rangle \langle I \rangle$, wherein $\langle U \rangle$ and $\langle I \rangle$ are respectively the time averaged voltage and the time averaged electric current of the imposed pulse shape. The total current as such is determined by the current density $j = \sigma E$ and the cross sectional area of the surface through which said current is measured.

For a description of the Dynamic Polarisation Process (DPP) according to the invention, the above-mentioned simplification that the current density goes beyond the time-independent, stationary state approximation given by $J = \sigma E$. Instead, we have to take into account all of the so-called Maxwell equations and allow time dependent polarisation effects. In this case the current density is given by:

$$j = \sigma E + dD/dt$$

where the dD/dt term is the time derivative of the molecular displacement field. D is related to the static polarisation vector of a dielectric medium with permittivity ϵ , by:

$$D = \epsilon E.$$

In the above indicated applications of systems based on PEF treatment the second term

in the equation is considered to be zero. At the pulse conditions where PEF systems operate in practise this is a valid assumption. However, in underlying application use is made of the action relying by the second term, i.e. dD/dt . It can be proven that with rapidly varying voltages through a capacitive coupling only the second term of the above mentioned equation is significant for a predetermined microbial effect. In contrast to the conducting current in the PEF-process, the displacement current in underlying application is only a reactive current which is present only temporarily after the initial polarisation of the medium.

The method according to the invention is now characterised by a number of the hereafter indicated characteristics:

1. the change of an the external imposed voltage generates an electrical field between two-capacitor plates which causes an temporarily voltage gradient across the product and across any present membrane structure;
2. the maximum of the imposed voltage should be sufficiently high to cause micro-biological inactivation. Typically field strengths are employed in excess of 1 kV/cm.
3. the external voltage has to imposed within a time interval smaller than the dielectrical relaxation time of the product given by its electrical conductivity and permittivity by $\tau = \epsilon / \sigma$,
4. the heat that is disposed in the product as a result of the polarisation-depolarisation cycles determined by the number of times the medium is polarised. In general, the energy density in a polarised medium is given by $u = 1/2 \epsilon E^2$. After each cycle of polarisation and depolarisation this equals the maximum energy contents which can be converted into heat. In case that a continuous wave (CW) oscillator of frequency ω is facilitated as the external voltage source, the heat dissipation per unit volume is determined by:

$$p = 1/2 \omega \epsilon \epsilon'' E^2, \text{ where } \epsilon'' \text{ is the so-called dielectrical loss factor.}$$

5. the heat development is caused primarily by the counter acted rotation (friction) of polar molecules and ions in the medium to be treated. For radio frequencies (RF) in the range of 1 to 1000 MHz these kind of losses are relatively small, for microwave (MW) frequencies in the range of 1-4 GHz these losses can be significantly.

By applying the method according to the invention products containing micro-

organisms and membrane structures can be treated in a completely different manner. It has been demonstrated that this method in principle can be used to stop bacterial spoilage at reduced temperatures and treatment times. An example of the performance of this novel method has been demonstrated and has been compared to a standard heat process (pasteurisation) that is commonly used by food manufactures and the pharmaceutical industry as a preservation method. The novel method can be applied as a mild preservation method for pre-packed products and has potential as a mild decontamination method of in shell eggs. Although this method has been evaluated for a pre-packed product, it is also applicable as an continuous process on a flow of bulk produce. To generalise its application: the method relies on dynamical polarisation cycles, induced by means of an external time dependant voltage source with specific requirements on the steepness of the rising edge of the imposed voltage shape rather than the duration of the imposed voltage. The basic differences between the dynamic polarisation processes according to underlying application and the prior art PEF-method are:

- use is made of a non-stationary electronic processes to generate an electrical voltage difference across a product;
- the coupling with the medium to be treated is capacitive;
- in principle no electrodes in physical contact with the product are required.

In contrast to the PEF-technology, the process according to underlying application functions in principle with cycles of continuously oscillating electrical fields. These can be applied by using electronic circuitry operating at a single resonant frequency. This is fundamentally different from the types of electronic circuitry employed in the PEF technology, where switches and PFN's have to be are operated over a spectrum of frequencies to obtain an electrical pulse.

1
CLAIMS

1. Method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in a treatment device comprising two electrodes connected to an electronic circuit such that in said product an electrical field is created, characterized in that the rise time or the leading edge of each imposed voltage pulse is shorter than the associated electronic relaxation time of the product under treatment which is defined by the ratio of electrical conductivity and permittivity of the product.

2. Method for treating products according to claim 1, where the cellular structures present in the bulk product are pathogenic or spoilage organisms and/or their spores, where treatment is applied as a mild preservation method to prevent the outgrowth of such organisms in the product after production during distribution or storage.

3. Method for treating products according to claim 1, where the cellular structures present or the bulk product contain target compounds as e.g. minerals, enzymes or molecular compounds which are exchanged at a higher rate through the membrane of such structures when applying the treatment.

4. Method for treating products according to the preceding claims, characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.

5. Method according to any of the preceding claims, characterised in that dependent on the type of product and target organisms contained in the product, the maximum field strength during a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target micro-organisms and or spores are functionally affected or inactivated leading into a shelf life stable, microbiologically safe product.

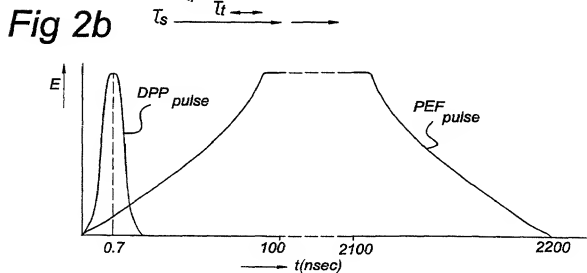
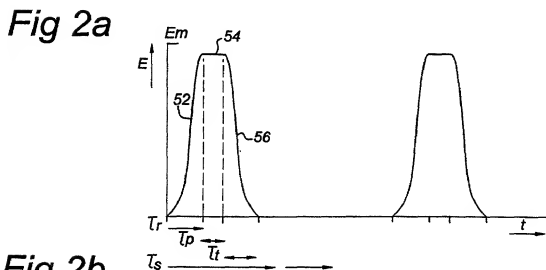
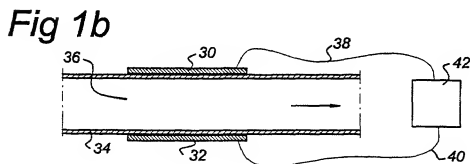
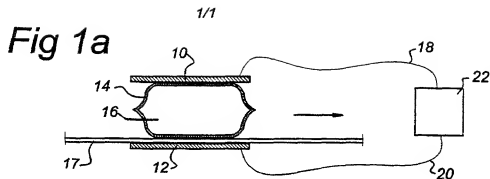
7. Method according to any of the preceding claims, characterised in that dependent on the type of product and the types of cellular structures contained in the product, the

maximum field strength reached in a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds with the bulk product.

5

7. Method according to any of the preceding claims, characterised in that the maximum field strength in the product during each pulse, the repetition frequency, the number of cycles and the total residence time in the process are selected such that the temperature of the product does not exceed a predetermined value during treatment.

10



COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL DESIGN, NATIONAL STAGE OF PCT OR CIP APPLICATION)

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Method for treating products by high voltage pulses

the specification of which: (complete (a), (b) or (c) for type of application)

REGULAR OR DESIGN APPLICATION

- a. ☐ is attached hereto.
b. ☐ was filed on _____ as Application
Serial No. _____ and was amended on _____
(if applicable)

PCT FILED APPLICATION ENTERING NATIONAL STAGE

- c. ☒ was described and claimed in International application No. PCY/NL00/00200
filed on 24 March 1999
and as amended on _____ (if any)

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a).

In compliance with this duty there is attached an information
disclosure statement 37 CFR 1.97

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code paragraph 119 of any foreign application (s) for patent of inventor's certificate listed below and have also identified below any foreign application for patent of inventor's certificate having a filing date before that of the application on which priority is claimed.

(complete (d) or (e))

- d. ☐ no such applications have been filed
e. ☒ such applications have been filed as follows

**EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION**

Country	Application Number	Date of filing (day, month, year)	Date of issue (day, month, year)	Priority claimed
The Netherlands	1011660	24 March 1999		Yes

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION**

CONTINUATION-IN-PART

(Complete this part only if this is a continuation-in-part application)

I hereby declare claim the benefit under Title 35, United States code, paragraph 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claim of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, paragraph 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

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As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric Jensen, Reg. No. 37,855, and Thomas W. PERKINS, Reg. No. 33,027 and Roland E. Long, Jr. Reg. No. 41,949 c/o YOUNG & THOMPSON, Second Floor, 745 South 23rd Street, Arlington, Virginia 22202.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date 7 September 2001

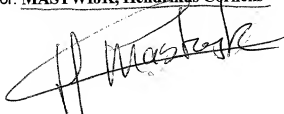
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